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STRUCTURAL BASIS FOR GENE REGULATION BY A THIAMINE PYROPHOSPHATE-SENSING RIBOSWITCH

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Genes are commonly turned on or off by protein factors that respond to cellular signals. The recent discovery of riboswitches proved that RNA molecules also control genes by directly sensing the presence of essential cellular metabolites. We determined the three-dimensional structure of the most widespread riboswitch class bound to its target, thiamine pyrophosphate, a co-enzyme derived from vitamin B1. These findings reveal how riboswitch RNA folds to form a precise pocket for its target and how a drug that kills bacteria tricks the riboswitch and starves disease-causing organisms of this essential compound.

RNA molecules, traditionally viewed as passive messengers of genetic information, have surprised researchers every few years with the discovery of novel functions that they perform. Recent studies have shown that some mRNAs - termed riboswitches - can sense changes in the levels of cellular metabolites and activate or repress genes involved in the biosynthesis and transport of these metabolites. Riboswitches are now recognized as one of the major metabolite-controlling systems that account for about 2% of genetic regulation in bacteria and that respond to various metabolites including co-enzymes, sugars, nucleotide bases, amino acids, and cations.

Riboswitches typically consist of two parts: a sensing region recognizing metabolites and an expression platform carrying gene-expression signals. Metabolite binding causes alternative folding in the sensing domain followed by conformational changes in the adjoining expression platform. This structural re-organization of the riboswitch results in the formation of specific structures that can terminate mRNA synthesis or prevent protein biosynthesis **(Figure 1)**. Remarkably, riboswitches do not need protein co-factors for recognition of their targets or for RNA folding. Though riboswitches are made of only 4 nucleotides instead of 20 different amino acids building the proteins, riboswitches can choose their target molecules among very similar metabolites as well as proteins do.

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In order to understand how riboswitches recognize their metabolite targets and regulate gene expression, we have determined the three-dimensional structure of the complex between thiamine pyrophosphate (TPP) and its cognate *E.coli* riboswitch at 2.05 Å resolution. The TPP riboswitches are the most widespread class of metabolite-sensing RNAs and the only riboswitches found in all three kingdoms of life. The structure shows that the riboswitch consists of two large helical domains and a short helix P1 connected by a junction **(Figure 2)**. The helical domains are parallel and contact each other by long-range tertiary interactions. TPP binds the riboswitch in an extended conformation and positions itself between and perpendicular to the helical domains such that opposite ends of TPP are bound to a specific RNA pocket. Notably, TPP carries negatively charged phosphate groups and the structure shows how RNA recruits positively charged metal ions to mediate otherwise unfavorable electrostatic interactions. Similar to purine riboswitches, TPP is largely enveloped in the structure of the complex and, in agreement with biochemical experiments, the TPP riboswitch folds upon binding to TPP. However, discrimination

between TPP and related compounds is achieved using a principle that is different from purine riboswitches. Purine riboswitches distinguish between adenine and guanine ligands via formation of Watson-Crick base pairs with either uridine or cytosine nucleotides located in strategic positions in the riboswitch. The TPP riboswitch, on the other hand, can be considered as a molecular ruler measuring the length of the ligand. Analogs of TPP lacking one or both phosphates cannot reach well into both binding pockets of the riboswitch. According to biochemical experiments, they cannot stabilize the metabolite-bound architecture of the riboswitch, including the key helix P1, and cannot effectively control gene expression. The central part of TPP is not specifically recognized by the riboswitch, and this observation explains how the man-made TPP-like drug pyrithiamine pyrophosphate, which differs from TPP in the middle part, targets the riboswitch and down-regulates expression of thiamine-related genes, thus starving microbes of TPP. Given the important role of riboswitches in various microorganisms and the fact that riboswitches have not yet been detected in the human genome, riboswitch structures should enable researchers to employ rational drug discovery strategies to create novel classes of antimicrobial compounds that specifically target riboswitches.

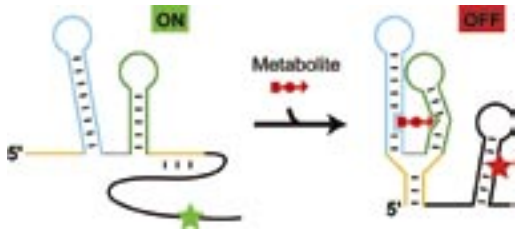

Figure 1. Schematic representation of the riboswitch's function exemplified by the thiamine pyrophosphate - specific riboswitch, which represses the initiation of protein biosynthesis in the presence of metabolite. The structural elements of the metabolite-sensing domain are colored in yellow, blue, green, and gray, and the expression platform is in black. In the absence of metabolite, the metabolite-sensing domain folds into the structure, which exposes the initiation signal of protein synthesis (green asterisk), thereby turning gene expression 'ON'. In the presence of metabolite (shown in red), the sensing domain folds into an alternative structure and causes the formation of the hairpin in the expression platform. As a result, the initiation signal becomes engaged in base pairing (red asterisk) and cannot function anymore, thereby shutting down gene expression, and acting as an 'OFF' switch.

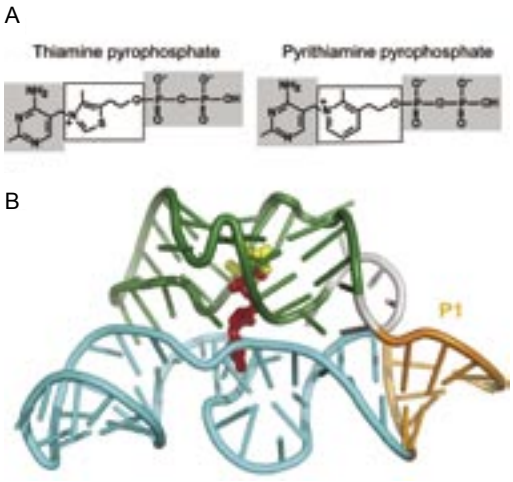

Figure 2. Structural models of a TPP riboswitch and its ligands. (A) Chemical structures of the natural metabolite TPP and the antimicrobial compound pyrithiamine pyrophosphate; (B) Crystal structure of the TPP-bound sensing domain from E.coli thiM gene in a ribbon representation. Structural elements of riboswitch are colored according to Figure 1: major helical domains are in green and blue, helix P1 in orange and three-way junction in gray. TPP and hydrated Mg cations are shown in red and yellow, respectively.